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Effects of Ground ULV Applications of Fenthion on Estuarine Biota II. Analytical Methods and Results.¹

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ABSTRACT

Methods of analyses were validated for quantitating fenthion residues in samples from two salt marsh sites subjected to ground ultra-low volume sprays. Concentrations of these residues were followed from the water's surface, through the water column and onto the sediment. For all sprays, the highest concentration, detected in the upper portion of the water column, was 0.48 $\mu\text{g/l}$ and occurred within the first hour after spraying. Detectable concentrations ($>0.010 \mu\text{g/l}$) of fenthion persisted in the water for up to 24 h. Fenthion did not accumulate to a detectable level ($0.010 \mu\text{g/g}$) in tissues of caged shrimp or fish.

INTRODUCTION

Techniques have been described for extracting and quantitating fenthion residues in freshwater (Hall and Kolbe, 1980, van Dyk et al., 1975; Dixon and Brust, 1971; Schaefer and Dupras, 1969), plants and animals (Anderson et al., 1966), rice (Aoki et al., 1975), fruits and vegetables (Ferreira and Fernandes, 1980), beef fat (Wright and Riner, 1979), corn and milk (Bowman and Beroza, 1968). In most

analytical methods, gas-liquid chromatography (GLC) with flame-photometric (FPD) or thermionic (TID) detectors was used to separate and quantitate fenthion residues. One multiresidue method for analysis of pesticides in water with silica gel cleanup was described in the "Manual of Analytical Methods for Analysis of Pesticides in Human and Environmental Samples" (Watts, 1980).

Since these techniques were not directly applicable to samples from our field research, quantitative extraction and cleanup methods were developed to measure fenthion in estuarine water and biota at concentration ranges expected following ultra-low volume (ULV) applications

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(0.01 to 100 $\mu\text{g/g}$ in water and 0.01 to 100 $\mu\text{g/g}$ in sediment and biota). The methods were then applied to analysis of the magnitude and duration of environmental concentrations of fenthion that existed following ground ULV applications. Analyses of water samples were employed to quantify exposures of nontarget species to fenthion. Residues in plants, sediments and laboratory test animals also were quantified to describe the fate and bioaccumulation of the pesticide. Fenthion deposited on the water's surface was quantified to determine amount of direct input into water. Such data are necessary to evaluate the significance of the biological responses and to describe the potential adverse environmental effects following ground ULV application of fenthion near salt marsh habitats.

MATERIALS AND METHODS

APPARATUS. A Hewlett-Packard model 5710 gas chromatograph² equipped with nitrogen-phosphorus detectors was used for analyses. Data were collected and analyzed qualitatively and quantitatively on a Hewlett-Packard 3357 laboratory computer system. Tissues and sediments were extracted in acetonitrile during homogenization in a Willems model 10-ST Polytron (Brinkman Instruments, Westbury, New York).

REAGENTS. All solvents were nano-grade (Burdick and Jackson Laboratories, Inc.) or equivalent. Silica gel ("Silica Gel 60," particle size 0.063-0.200 mm) was obtained from E. Merck, Darmstadt, West Germany. Reagent grade glacial acetic acid (Baker) was used to make the 1% (v/v) acetic acid in hexane for silica gel column conditioning and elution.

STANDARDS. A fenthion standard (Lot C-102, 97% pure) was obtained from the U.S. EPA Pesticide Repository (Las Vegas, Nevada). Primary standard (1.0 mg/ml) was prepared in p-xylene, working standards (0.040 ng/ μl) in hexane. Sediment and biota were fortified with fenthion dissolved in acetone; however, hexane solutions of fenthion were used in determining recoveries from silica gel columns.

PROCEDURES. One-liter water samples were collected in 1.0 l graduated borosilicate glass bottles (Wheaton); after 100 ml of petroleum ether were added, the

bottles were stored in styrofoam containers packed with ice and transported to the laboratory for extraction and quantitation. Samples usually were analyzed within 24 h after being received; however, unrefrigerated seawater samples fortified with 1.0 μg of fenthion/l were stored for 4 days without measurable loss of fenthion residues. Seawater samples were extracted twice with 100 ml petroleum ether. The solvent was evaporated to 10 ml on a steam cabinet and concentrated to 1 ml with a gentle stream of nitrogen at 35°C.

Tissue, sediment and *Juncus* grass (*Juncus roemerianus*) samples were wrapped in foil, stored on ice, transported to the laboratory, and frozen at -4°C until analyzed (usually within 7 days). Animal and sediment samples were prepared for analysis by placing from 1 to 8 g of material in a 150 mm by 25 mm screw-cap culture tube and extracting with acetonitrile by homogenization with a polytron. One to three grams of *Juncus* grass were weighed into a 150 mm by 25 mm screw-cap test tube and fenthion residues extracted by the same procedure.

Surface samplers adsorbed fenthion droplets at the water's surface following ULV sprays. These samplers were made of 7 mm thick acrylic plastic (25 cm by 25 cm base with 2 cm high sides) attached to 2.5 cm thick styrofoam, and contained filter paper that could be removed for subsequent analysis of fenthion residues. During the first three sprays, Whatman No. 3 filter paper, 24.0 cm diam., was used in the samplers. Whatman No. 44 was used during Spray 4 since this filter paper required less cleanup before use. The samplers were constructed to expose only 269 cm^2 of filter paper, and this value was used in all calculations. The samplers were floated on the surface of the water prior to spraying and collected 15 to 30 min after spraying. The filter paper was removed from the samplers and placed in screw-cap glass bottles; 50 ml of petroleum ether were added and the contents shaken for 30 seconds before the samples were transported to the laboratory. Two additional 50-ml extractions were completed within 24 h after samples reached the laboratory and residues were determined and expressed as ng/ cm^2 .

Cleanup columns were prepared by adding 3.5 g silica gel, followed by 2.0 g of

anhydrous sodium sulfate (granular), to a 200 mm by 9 mm i.d. Chromaflex® column (Kontes Glass Co., Vineland, NJ) plugged with glass wool. Silica gel was stored at 130° C, deactivated by addition of 20% (w/w) distilled water, and tumbled on a roto rack for 2 h prior to use. Fenthion eluted with 20 ml of 5% (v/v) ethyl ether in hexane. Fenthion residues were concentrated to not less than 1.0 ml with a gentle stream of nitrogen.

QUALITY ASSURANCE. Before spraying began, water samples were collected at each sampling site. These samples were subdivided into two groups: field blanks and field spikes. One-liter field blank samples were analyzed to determine if water and glassware were contaminated with fenthion. One-liter water samples were fortified with 0.21 µg fenthion at the sampling site to determine the stability of fenthion from time of sampling to analysis in the laboratory. Another one-liter seawater sample from the laboratory was fortified with fenthion and analyzed with each group of field samples to determine percentage recovery of fenthion during analyses. These concentrations ranged from 0.10 µg to 1.0 µg fenthion/l water. Percentage recovery of fenthion from silica-gel-column cleanup was determined by fortifying one cleanup column with 1.0 µg fenthion with each set of samples. One blank filter paper was fortified with 1.0 µg fenthion and placed in screw-top glass bottles; 50 ml petroleum ether added and contents shaken for 30 seconds. Sample bottles were placed in a cooler with ice and transported to the laboratory.

RESULTS

Recoveries from all substrates fortified with fenthion exceeded 85% at all fortification levels, including method detection limits (0.010 µg/l and 0.010 µg/g (Lores et al., 1985). Minimum detection limit is defined as the minimum quantifiable amount that has: 1) no interference from coextractives in a sample matrix, 2) is within linear range of the nitrogen-phosphorus detector, 3) is at least one quarter of the peak height of the standard (0.040 ng/ml, 5 µl injection), and 4) is recovered during the extraction and cleanup with efficiency greater than 85%. Fenthion residues in fish and shrimp in laboratory tests were

still detectable 48 h after exposure to 2.0 µg/l. Detectable residues of fenthion accumulated in shrimp even though deaths occurred within 12 h of exposure. The methods were developed for application to samples from actual ULV sprays of fenthion, however laboratory tests helped to validate the analytical methods.

WATER SAMPLES. Results of time-series water samples collected at Sites 1 and 2 and analyzed for fenthion residues are shown in Tables 1 and 2. A key to the station location is included in Figures 1 and 2 in Clark et al. (this issue). Changes in fenthion concentration over time at a sampling site resulted from a combination of various mixing actions within the lagoons with tidal exchange.

At Site 1 (Table 1), no extreme differences in fenthion concentrations occurred during the first 3 sprays. Maximum concentrations were 0.28 µg/l in Spray 1, 0.68 µg/l in Spray 2 and 0.47 µg/l in Spray 3. A typical time-concentration profile that resulted from the sprays at Sites 1 and 2 is shown in Figure 1A. At Site 1, where there was sufficient depth for vertical concentration profiles to develop, fenthion passed through the water column to middle and bottom sample depths with smaller maximum concentrations caused by dilution (Figure 1B). Maximum concentrations with time for each depth location were: Top = 0.23 µg/l at 0.75 h, Mid = 0.13 µg/l at 1.0 h and Bottom = 0.15 µg/l at 1.0 h. Wind speed and direction were different for all sprays and may account for the differences in initial fenthion concentrations and variability between sampling stations. The light, southerly wind during Spray 2 permitted the highest concentrations of fenthion in water and most effective mosquito kill. Tidal flushing was an important factor in the residence time for detectable concentrations of fenthion at Site 1. Spray 1 occurred 1 h post-flood tide, Spray 2 was 3 h after pesticide application. Fenthion was detected for the longest period post-spray following Spray 2. Concentrations of fenthion in water at Site 1 following Spray 4 were different from all other sprays. There were relatively strong southerly winds at the time of Spray 4. Fenthion concentrations decreased below detection limits within an hour.

Table 1. Fenthion concentrations ($\mu\text{g/l}$) in water at Site 1^a. Subscript designations refer to depth at which samples were taken: T = Top, M = Mid Water Column, B = Bottom. ND = none detected at 0.010 $\mu\text{g/l}$ detection limit. Data for Spray 1 represent the average of duplicate samples, all others are from a single grab. Refer to Clark et al. (this issue) for sample station location.

Sample Time (hours)	Spray 1				Spray 2				Spray 3							
	Station				Station—Depth				Station—Depth							
	A _T	B _T	C _T	A _T	B _T	C _T	D _T	E _T	E _M	E _B	A _T	A _M	A _B	B _T	B _M	C _T
0.25	0.064			ND	0.54		0.26	0.68			0.047	0.048	0.016	0.24	0.067	0.055
0.50	0.28			0.16	0.17	0.36	0.12	0.28			0.20	0.021	ND	0.21	0.066	0.031
0.75											0.23		ND	0.27	0.090	0.044
1	0.053			ND	0.070	0.42	0.020	0.48			0.16	0.13	0.15	0.47	0.10	0.045
1.5											0.11	0.026	0.12	0.12	0.051	0.051
2	ND ^b	0.059 ^b	0.083 ^b	0.040	0.018	0.14	0.033	0.28	0.12	0.17	ND	0.027	0.014	0.054		0.075
2.5																
3	ND			0.12	0.11	0.17	0.13	0.13			ND	ND	0.024	0.016		0.050
3.5																
4	ND			0.11	0.12	0.14	0.11	0.071	0.11	0.11						
5	ND															
5.5				0.11	0.096	0.10	0.11	0.12	0.079	0.053	0.018	ND	ND			0.023
6	0.014															
8	ND															
10	ND															
12	ND ^b	0.014 ^b	ND ^b	0.024	0.019	0.020	ND	0.012	0.020	0.047	ND	ND	0.018	ND		ND
18	ND	ND	ND	ND	ND	ND	ND	ND			ND			ND		ND
24	ND ^b	ND ^b	ND ^b	ND	ND	ND	ND	ND			ND			ND		ND

^aOnly three water samples contained detectable residues of fenthion after spray 4: 0.25 h, C_T = 1.10; 0.50 h B_M = 0.011 and C_T = 0.017.

^bDuplicate samples analyzed with average concentrations reported.

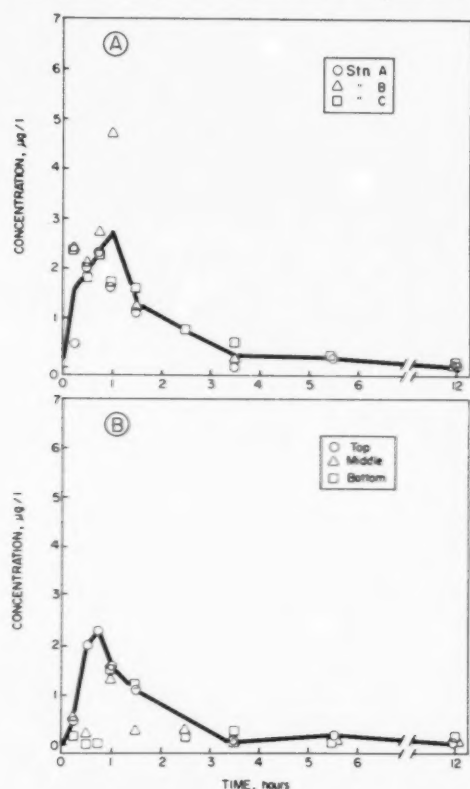


Fig. 1. Concentration of fenthion in water at Site 1 after Spray 3. In graph A, the line represents average concentrations at the top for each sampling at stations A, B and C. In graph B, the line represents concentrations at the top for station A with a depth profile of middle and bottom samples also shown.

At Site 2 (Table 2), concentrations of fenthion in water did not vary considerably between Sprays 2 and 3, whereas concentrations from Spray 1 were lower during the first 2 h. Maximum concentrations from Spray 1 were $0.061 \mu\text{g/l}$, compared to $0.38 \mu\text{g/l}$ for Spray 2 and $0.48 \mu\text{g/l}$ for Spray 3. Rapid disappearance of fenthion at station A was probably due to its shallow depth (< 3 inches at low tide) and silty substrate. Since all depths at Site 2 were less than 0.50 m, no attempt was made to stratify sampling at various depths. During Spray 4, the relatively greater distance between the spray truck and sampling stations at Site 2 (50 m), compared to Site 1 (10 m), allowed pesticide to be deposited in the water at Site 2, rather than having it blown over the top of the site. Maximum

concentration was $0.40 \mu\text{g/l}$ and fenthion remained detectable at this site for 6 h.

SURFACE SAMPLES. Droplet deposition on filter papers floated on the selected water surface sites was measured during each spray. These samples were collected and analyzed to determine if fenthion actually reached the water surface, and determine if fenthion was carried into the marsh grass. These data were used as indicators of pesticide contacting water surface, not as precise measurement of total pesticide reaching the surface.

For truck-mounted ULV sprays, concentrations of fenthion from sprays 1 to 4 ranged from 1.3 to 14 ng/cm^2 (Table 3). Distance from the road to the pond was a factor in the amount of fenthion deposited when adverse wind conditions and thermals caused the droplets to drift away from the site. As observed at Site 2 during Spray 1 and Site 1 during Spray 3, wind from directions that do not carry the spray droplets on a direct path ($< 45^\circ$ from the direction of the spray nozzle) to the target area deposit considerably less pesticide. Thermals also decreased the number of droplets that settled onto the water surface because the droplets were transported past the sites closer to the road and deposited more densely at sites farther from the road (Spray 4). Highest concentrations of fenthion in water following truck-mounted ULV sprays occurred on evenings when slight winds (< 5 mph) blew directly into the pond area (both sites Spray 2; Site 1, Spray 1).

RESIDUES IN BIOTA AND SEDIMENT. *Juncus* grass samples were taken along the water's edge after Sprays 1 and 2. Only three *Juncus* grass samples nearest the sprayer contained detectable residues. Concentrations of fenthion ranged from 0.013 to $0.050 \mu\text{g/g}$. These data were used as indicators of pesticide contact not as quantitative distribution of pesticide throughout marsh grasses.

Field-exposed animals were caged in mesh containers that floated on the surface and extended to a depth of 0.3 to 0.5 m (Ref. Borthwick and Stanley, this issue). Samples were collected at 12 -, 24 -, and 48 -h post-spray. Fish or shrimp exposed in the field did not accumulate detectable concentrations of fenthion. Residues of fenthion were not detected in the upper 5

Table 2. Fenthion concentrations ($\mu\text{g/l}$) in water at Site 2. (Refer to Table 1 for key to subscripts and sampling information and to Clark et al. (this issue) for sample station locations).

Time (hours)	Spray 1			Spray 2			Spray 3			Spray 4 ^b		
	A _T	B _T	C _T	A _T	B _T	C _T	A _T	B _T	C _T	A _T	B _T	C _T
0.25	ND	ND	ND			0.17	0.10	0.40	0.30	0.031	0.17	0.20
0.50	ND	ND	ND			0.38	0.48	0.17	0.19	0.023	0.40	0.16
0.75							0.41	0.14	0.22	0.049	0.16	0.21
1		ND				0.28	0.27	0.077	0.11	0.014	0.054	0.14
1.5								0.023	0.11	0.011	0.062	0.11
2	0.061 ^a	0.011 ^a	ND ^a			0.24						
2.5							ND	0.032	0.10	ND	0.025	0.062
3		0.021				0.16	ND		0.032	ND	0.013	0.034
3.5												
4		0.032				0.21						
4.5												
5		ND										
5.5												
6		0.023				0.13	ND	0.016	0.015	ND	ND	0.018
8		ND										
10		ND										
12	ND ^a	0.011 ^a	ND ^a			0.16	ND	ND	ND			
18	ND	ND	ND			0.044	ND	ND	ND			
24	ND ^a	ND ^a	ND ^a	0.028		0.036	ND	ND	ND			

^aDuplicate samples analyzed; average concentration is reported.^bSampling was discontinued after 6 h since concentration of fenthion had diminished.

Table 3. Residues of fenthion (ng/cm²) deposited on filter papers in samplers floating on the water surface at various distances from the ULV spray generator. (Refer to Clark et al. (this issue) for sample station locations).

Site	Spray	Location of samplers relative to water sample stations									
		Road	Station A		Station B			Station C		Sound	
		1	2	3	4	5	6	7	8	9	10
1	1	14	— ^a	9.2	—	—	2.8	—	—	4.6	—
	2	3.9	3.8	12	4.6	3.2	6.0	5.1	—	5.1	5.9
	3	ND	ND	ND	—	—	ND	—	—	ND	ND
	4	—	ND	—	ND	ND	ND	—	—	ND	ND
2	1	ND	—	1.6	—	—	ND	—	—	ND	—
	2	4.3	—	—	—	—	—	—	—	6.7	—
	3	1.3	—	2.1	—	1.7	ND	—	ND	ND	ND
	4	ND	1.5	ND	—	ND	—	ND	—	5.0	ND

^aSurface samplers not deployed here.

to 10 mm of sediment samples taken after Spray 1 at the 24-h sampling period.

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